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Synthesis of 1-O-Acylglycerol 2,3-Cyclic Phosphate: Determination of the Absolute Structure of PHYLPA, A Specific Inhibitor of DNA Polymerase α

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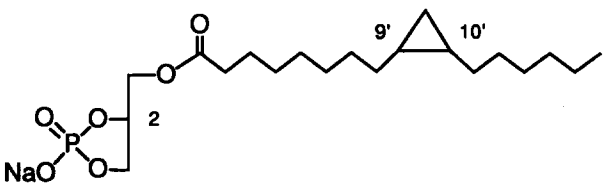
Key Words: PHYLPA; DNA polymerase α; lysophosphatidic acid; cyclic phosphate; cyclopropane; tris(triazole) phosphate.

Abstract: *Four possible stereoisomers of PHYLPA, a specific inhibitor of DNA polymerase α, were synthesized in enantioselective manners. These isomers were examined for inhibition activity for DNA polymerase α, and the structure of PHYLPA was established as sodium 1-O-[(9'S, 10'R)-9',10'-methanohexadecanoyl]-sn-glycerol 2,3-cyclic phosphate.*

PHYLPA[1] was recently isolated as a specific inhibitor of DNA polymerase α from myxoamoebae of a true slime mold, *Physarum polycephalum*. Structural study by MS, IR, and NMR has lead to the assignment of PHYLPA as 1-*O*-(9',10'-methanohexadecanoyl)glycerol 2,3-cyclic phosphate, although the absolute configurations at C(2), C(9'), and C(10') have not been yet established.[2]

Scheme 1



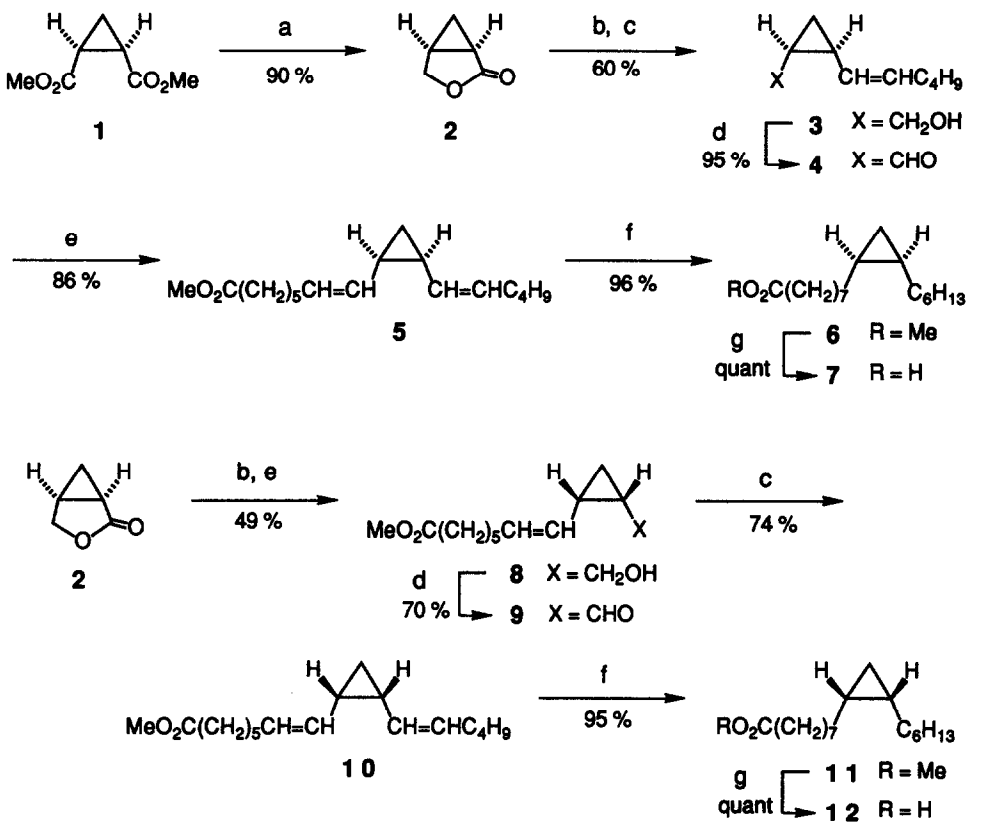
PHYLPA

PHYLPA is quite interesting because (1) it exhibits opposite effects on cell proliferation and DNA synthesis[1] compared to lysophosphatidic acid (LPA) and phosphatidic acid (PA) of current biological interests,[3] and (2) these difference is derived by the novel structure which involves the glycerol cyclic phosphate and cyclopropane-containing hexadecanoic acid,[4] and that (3) to our knowledge, only diterpene antibiotic aphidicolin[5] was known as a specific inhibitor of DNA polymerase α. This paper describes the stereocontrolled synthesis of four possible stereoisomers of PHYLPA and the determination of the absolute structure of PHYLPA by examining their inhibition activity for DNA polymerase α.

Our synthetic route to PHYLPA is rather straightforward involving the initial coupling of cyclopropane-containing hexadecanoic acid with isopropylidene glycerol, followed by deacetalization and final transformation to a cyclic phosphate.

Preparation of both enantiomer of 9,10-methanohexadecanoic acid, **7** and **12**, is summarized in Scheme 2. The chiral synthon employed in the present study was the bicyclic γ-lactone (+)-**2**, readily prepared from *meso* diester **1** by enzymatic approach.[6] The γ-lactone **2** was reduced to hemiacetal with DIBAL (1.05 equiv), and the hemiacetal, without purification, was reacted with pentylidenetriphenylphosphorane in DMSO[7] to obtain the vinyl cyclopropane **3**[8,9] in 60% yield from **2**. The alcohol **3** was oxidized with PCC, and the resulting aldehyde **4** was then reacted with (6-carboxyhexylidene)triphenylphosphorane[10] in DMSO, followed by the treatment with diazomethane in Et_2O to give the diene **5** in 86% yield.

Scheme 2



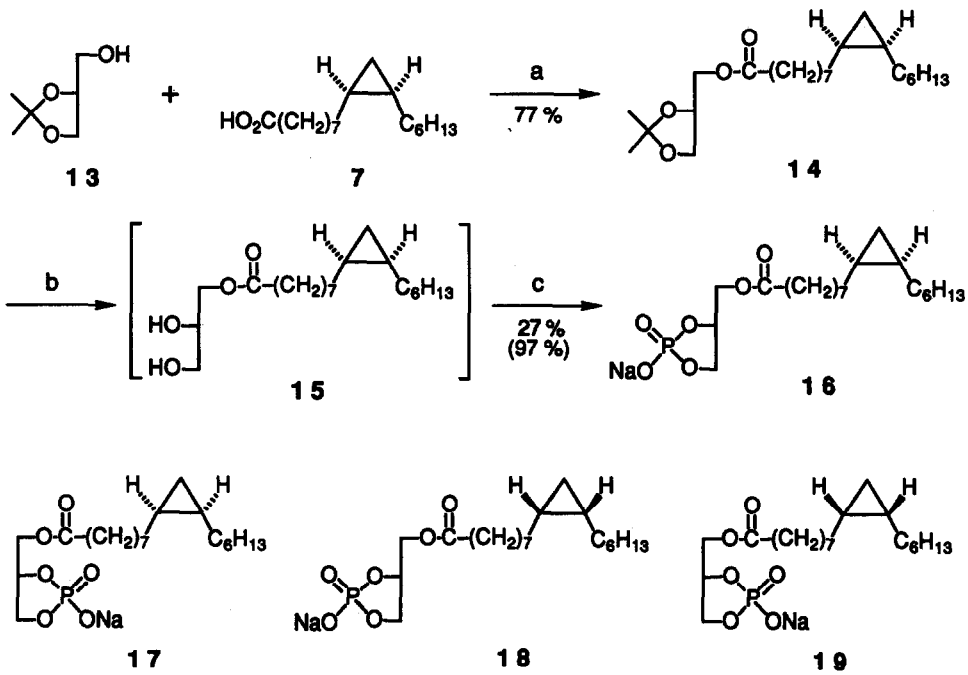
Reagents and conditions: (a) (i) PLE, pH 8.0 phosphate buffer, r.t., 24hr; (ii) BH_3·SMe_2, $B(OMe)_3$, THF, -20°C→r.t., 24hr; (iii) p-TsOH, benzene, reflux, 30min: (b) DIBAL, CH_2Cl_2/hexane, -78°C, 1hr: (c) $Ph_3P^+C_5H_{11}$ Br^-, $NaCH_2SOCH_3$, DMSO, r.t., 1hr: (d) PCC, NaOAc, MS3A, CH_2Cl_2, r.t., 1hr: (e) (i) $Ph_3P^+C_6H_{12}CO_2H$ Br^-, $NaCH_2SOCH_3$, DMSO, r.t., 1hr; (ii) CH_2N_2, Et_2O: (f) $KO_2CN{=}NCO_2K$, AcOH, MeOH, reflux, 8hr: (g) NaOH, THF/H_2O, reflux, 4hr.

Hydrogenation of the diene **5** was successfully achived by diimide reduction[11,12] (generated *in situ* from $KO_2CN{=}NCO_2K$ and AcOH) to provide **6**[13] in excellent yield. Finally, (9*S*,10*R*)-9,10-methanohexadecanoic acid (**7**) was obtained in quantitative yield by alkaline hydrolysis of **6**. The enantiomeric **12** was also synthesized from γ-lactone **2** by simply reversing the order of the phosphoranes. (Scheme 2)

Cyclopropane-containing hexadecanoic acid **7** was then coupled with (*R*)-glycerol acetonide **13**[14] to afford **14** in 77% yield. The transformation of **14** to cyclic pohosphate **16** was carried out without purification of the diol **15**. Thus, the acetonide **14** was treated with PPTS in MeOH at 50°C for 30 min,[15] and the mixture of **15**[16] and unchanged **14** was reacted with tris(1,2,4-triazole) phosphate[17,18] to obtain the cyclic phosphate **16**[19] in 27% yield (97% yield based on the recovered **14**) as a white powder after lyophilization.

Isomeric **17**, **18**, and **19** were also synthesized in a similar manner.[20] Determination of the structure (relative stereochemistry) by spectroscopic analysis was found difficult because ^{1}H-NMR and ^{13}C-NMR spectra of **16** (**19**), **17** (**18**), and natural PHYLPA were indistinguishable.

Scheme 3



Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, r.t., 12hr ; (b) PPTS, MeOH, 50°C, 0.5hr; (c) (i) tris(1,2,4-triazole) phosphate, THF, 0°C, 20min, (ii) 2%HCl, (iii) NaH, Et_2O.

Inhibition activity of each isomer for immunoaffinity-purified calf thymus DNA polymerase α was also examined, and only **16** exhibited the comparable activity as natural PHYLPA. Relative activities of other isomers compared to natural PHYLPA were estimated to be *ca* 1/3, <1/10, and <1/10 for **17**, **18**, and **19**, respectively. Therefore, the structure of PHYLPA was determined to be **16** (sodium 1-*O*-[(9'*S*,10'*R*)-9',10'-methanohexadecanoyl]-*sn*-glycerol 2,3-cyclic phosphate). Other quite interesting finding is that no inhibition activity was observed for demethano derivative (1-*O*-hexadecanoylglycerol 2,3-cyclic phosphate). Other

biological experiments such as cell proliferation using synthetic cyclic phosphates are now in progress, and results will be reported in due course.

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8. All new compounds gave satisfactory ^{1}H-NMR, ^{13}C-NMR, IR, and MS spectra.
9. Wittig reaction described herein produced cis- and trans isomers in favor of cis isomers. (~9:1 estimated by ^{1}H-NMR spectra) Isomeric mixtures were used for further transformations without separation.
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12. Catalytic hydrogenation of vinylcyclopropanes with PtO_2 pretreated with hydrogen resulted in a product containing a considerable amount of hydrogenolysis product of the cyclopropane ring. Separation of these mixture by chromatography or by distillation were found difficult. See also; Meinwald, J.; Labana, S. S.; Chadha, M. S. *J. Am. Chem. Soc.* **1962**, *85*, 582-585.
13. **6**; $[\alpha]_D^{25}$ +0.19°(*c* 8.0, $CHCl_3$); ^{1}H-NMR (400MHz, $CDCl_3$) δ -0.33 (1H, ddd, *J*=4.0, 4.0, 4.0Hz), 0.56 (1H, ddd, *J*=4.0, 7.5, 7.5Hz), 0.64 (2H, m), 0.89 (3H, t, *J*=7.0Hz), 1.09-1.62 (22H, m), 2.30 (2H, t, *J*=7.5Hz), 3.67 (3H, s). **11**; $[\alpha]_D^{20}$ -0.10°(*c* 5.4, $CHCl_3$).
14. (*R*)- and (*S*)-glycerol acetonide were prepared from (*R*)- and (*S*)-*O*-benzylglycidol, respectively; (i) 1*N* NaOH/H_2O-*t*-BuOH, (ii) *p*-TsOH/acetone, and (iii) Li, NH_3/THF.
15. Prolonged reaction time and/or the employment of other conditions such as AcOH/THF-H_2O or 1*N* HCl-THF resulted in the cleavage of both isopropylidene and 1-*O*-acyl groups.
16. Acyl migration did not occur during deprotection, which was confirmed by converting the diol to the corresponding bis-(*R*)-MTPA ester.
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18. Other phosphorylating reagents such as diphenyl chlorophosphate did not give cyclic phosphate. For review, see; Ramirez, F.; Marecek, J. F. *Synthesis* **1985**, 449-488.
19. ^{1}H-NMR (400MHz, $CDCl_3$/CD_3OD (3/1)) δ -0.33 (1H, ddd, *J*=4.0, 4.0, 4.0Hz), 0.57 (1H, ddd, *J*=4.0, 7.5, 8.5Hz), 0.64 (2H, m), 0.89 (3H, t, *J*=7.0Hz), 1.10-1.80 (22H, m), 2.36 (2H, t, *J*=7.5Hz), 3.97 (1H, ddd, *J*=7.0, 9.0, 9.0Hz), 4.20 (1H, dd, *J*=5.0, 12.0Hz), 4.25 (1H, dd, *J*=6.0, 12.0Hz), 4.28 (1H, ddd, *J*=6.2, 9.0, 12.8Hz), 4.59 (1H, ddddd, *J*=5.0, 6.0, 6.0, 6.2, 7.0Hz).
20. Yields (not optimized) for each isomers are as follows [*O*-acylglycerol acetonide, cyclic phosphate (based on the recovered acetonide]: **17** [67%, 25% (70%)]; **18** [81%, 25% (70%)]; **19** [77%, 26% (90%)].

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